Thermo SCIENTIFIC

PRODUCT INFORMATION

DpnI

#ER1705 1000 U

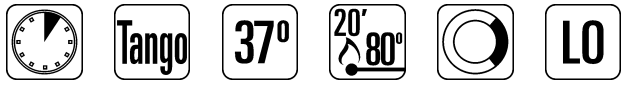
Lot: ___ Expiry Date: _

5'...**G m6A↓ T C** ...3'

3'...**C T↑m6A G** ...5'

Concentration: 10 U/µL
Source: *E.coli* that carries the cloned *dpnIR* gene from *Diplococcus pneumoniae* G41
Supplied with: 1 mL of 10X Buffer Tango

Store at -20°C



BSA included

www.thermoscientific.com/onebio

RECOMMENDATIONS

1X Thermo Scientific Tango Buffer (for 100% DpnI digestion)

33 mM Tris-acetate (pH 7.9), 10 mM magnesium acetate, 66 mM potassium acetate, 0.1 mg/mL BSA.

Incubation temperature

37°C.

Unit Definition

One unit is defined as the amount of DpnI required to digest 1 µg of pBR322 DNA (*dam* methylated) in 1 hour at 37°C in 50 µL of recommended reaction buffer.

Dilution

Dilute with Dilution Buffer (#B19): 10 mM Tris-HCl (pH 7.4 at 25°C), 100 mM KCl, 1 mM EDTA, 1 mM DTT, 0.2 mg/mL BSA and 50% glycerol.

Double Digests

Tango™ Buffer provided simplifies buffer selection for double digests. 98% of Thermo Scientific restriction enzymes are active in a 1X or 2X concentration of Tango Buffer. Please go to www.thermoscientific.com/doubledigest to choose the best buffer for your experiments.

Storage Buffer

DpnI is supplied in: 10 mM Tris-HCl (pH 7.4 at 25°C), 400 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.2 mg/mL BSA and 50% glycerol.

Recommended Protocol for Digestion

- Add:

nuclease-free water	16 µL
10X Buffer Tango	2 µL
DNA (0.5-1 µg/µL)	1 µL
DpnI	0.5-2 µL

- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours.

The digestion reaction may be scaled either up or down.

Thermal Inactivation

DpnI is inactivated by incubation at 80°C for 20 min.

ENZYME PROPERTIES

Enzyme Activity in Thermo Scientific REase Buffers, %

B	G	O	R	Tango	2X Tango
100	100	50-100	50-100	**100**	50-100

Methylation Effects on Digestion

Dam: does not cut *dam*⁻ DNA.
Dcm: never overlaps – no effect.
CpG: may overlap – no effect.
EcoKI: never overlaps – no effect.
EcoBI: may overlap – effect not determined.

Stability during Prolonged Incubation

A minimum of 0.1 units of the enzyme is required for complete digestion of 1 µg of pBR322 DNA in 16 hours at 37°C.

Number of Recognition Sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
116	0	22	15	15	15	7

For **CERTIFICATE OF ANALYSIS** *see* back page

Note

- DpnI requires the presence of N6-methyladenine within the recognition sequence to cleave DNA.
- DNA purified from a *dam*$^{+}$ strain will be a substrate for DpnI.
- DpnI will only cleave fully-adenomethylated *dam* sites. Hemi-adenomethylated *dam* sites DpnI cleaves 60X more slowly.
- DpnI, Bsp143I and MboI all recognize the same sequence but have different methylation sensitivities and cleavage sites.

CERTIFICATE OF ANALYSIS

Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 160-fold overdigestion with DpnI (10 U/µg pBR322 DNA x 16 hours).

Ligation and Recleavage (L/R) Assay

The ligation and recleavage assay was replaced with LO test after validating experiments showed LO test ability to trace nuclease and phosphatase activities with sensitivity that is higher than L/R by a factor of 100.

Labeled Oligonucleotide (LO) Assay

No detectable degradation of single-stranded or double-stranded labeled oligonucleotides occurred during incubation with 10 units of DpnI for 4 hours.

Quality authorized by:  Jurgita Zilinskiene

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively *for research purposes and in vitro use only.* The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Please refer to www.thermoscientific.com/onebio for Material Safety Data Sheet of the product.